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Genetic and technological characterization of lactic acid bacteria isolated from tropically grown fruits and vegetables

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ABSTRACT

Phyllosphere microorganisms are common contaminants of fruit or vegetable containing foods. The aim of this study was to identify and characterize lactic acid bacteria isolated from fruits and vegetables from Reunion Island, regarding possible application in food. Among 77 isolates, a large diversity of species was observed, with isolates belonging to *Lactobacillus plantarum* (3 isolates), other species of *Lactobacillus* (3), *Lactococcus lactis* (13), *Leuconostoc pseudomesenteroides* (25), *Leuconostoc lactis* (1), *Leuconostoc mesenteroides* (7), *Leuconostoc citreum* (14), *Weissella cibaria* (4), *Weissella confusa* (4), other species of *Weissella* (2) and *Fructobacillus tropaeoli* (1). Several of these species, although belonging to lactic acid bacteria, are poorly characterized, because of their low occurrence in dairy products. *Lactobacillus*, *Lactococcus*, *Leuconostoc* and *Weissella* isolates were classified by $(GTG)_5$ fingerprinting in 3, 6, 21 and 10 genetic groups, respectively, suggesting a large intra-species diversity. Several *Weissella* and *Lactobacillus* isolates were particularly tolerant to acid and osmotic stress, whereas *Lc. pseudomesenteroides* 60 was highly tolerant to oxidative stress. Isolates of *Weissella* 30, 64 and 58, *Leuconostoc* 60 and 12b, *Lactobacillus* 75 and *Fructobacillus* 77 present relevant characteristics for their use as starters or as preservative cultures for fruits and vegetables.

1. Introduction

For food made from fruits and vegetables, raw material carries numerous microorganisms on its phyllosphere, including both Gram negative and positive bacteria, yeasts and molds, which diversity and number depend on agricultural practices, water quality, environmental conditions, ripening stage and seasons (Leff and Fierer, 2013). Fresh fruits or vegetables may carry foodborne pathogens, resulting into outbreaks (Ramos et al., 2013; Telias et al., 2011). Yeast, molds or bacteria development result in spoilage of minimally processed foods from plant origin (Francis et al., 2012). In recent years, metagenomic studies investigated sources and contamination routes of fresh fruits and vegetables (Alegbeleye et al., 2018; Droby and Wisniewski, 2018; Vepštaitė-Monstavičė et al., 2018). It was demonstrated that raw fruits and vegetables were a considerable way of bacterial contamination in canned and ready-to-eat foods (Durand et al., 2015; Guinebretiere et al., 2003; Pothakos et al., 2014). Hopefully raw material contamination not only carries undesirable microorganisms but also bacteria that are useful for food processing or beneficial to food quality. For instance, a dominant lactic acid bacteria (LAB) isolated from tomato surface had inhibitory activities against natural microbial population growth on tomato purée and could be considered as a biological method to control the proliferation of contaminants (Sajur et al., 2007).

LAB contribute to food quality thanks to their fermentative activity, their biopreservation or their probiotic properties. They are characterized by a fast growth under moderately acidic and anaerobic conditions and as such are well-adapted for growth in fruit- or vegetable-based foods. Consequently, LAB are frequently detected in traditional spontaneous fermented foods, including fruits- and vegetables-based food (Tamang et al., 2016). During spontaneous fermentation, LAB are dominant and prevent the growth of potential spoilage and pathogenic microorganisms, enhancing the safety and shelf-life of food. These bacteria produce several interesting compounds, such as bacteriocins, vitamins, exopolysaccharides and enzymes, which modify food composition and properties.

However, LAB represent a minority part of the autochthonous initial microbiota of fruit and vegetable phyllosphere (Leff and Fierer, 2013). *Lactobacillus*, *Leuconostoc*, *Weissella*, *Enterococcus* and *Pediococcus* are the LAB genera the most frequently isolated from raw fruits and vegetables (Di Cagno et al., 2013). These genera are also the most frequent in spontaneously fermented fruits and vegetables. The role and the succession stage of LAB involved in food from plant origin fermentation

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are not clearly understood, especially for *Weissella* spp. (Fessard and Remize, 2017). Since LAB are naturally present on fruit surface and produce several antimicrobial compounds, their use as biological agent to control and prevent the growth of undesirable microorganisms without change of sensory properties of food is also considered. A better knowledge of LAB from fruits and vegetables constitutes thus an important step for the development of starter or preservative cultures for fruit- and vegetable-based foods. Moreover, the phenotypic diversity of LAB species is well documented for dairy applications but much less for plant origin foods or beverages. Hence, characterization of the autochthonous LAB population from raw fruits and vegetables deserves a deeper investigation.

This study describes the identification and the characterization of LAB isolated from fruits and vegetables of Reunion Island. Papaya, tomatoes and pickled cabbage were sampled. Pickled cabbage, called "achards" at Reunion Island, is a mix of vegetables (white cabbage, carrots, green beans, chili pepper) with vinegar, salt, curcuma and ginger, which arbours high levels of LAB (Fessard et al., 2016). Molecular biology methods such as *16S rRNA*, *pheS* and *recA* gene sequencing and $(GTG)_5$ fingerprinting were used to identify and classify LAB. LAB from different genetic groups were further characterized phenotypically, with the examination of production of EPS, the influence of temperature on the growth and tolerance to acid, salt, bile salts or hydrogen peroxide in the view of possible use in the food industry.

2. Materials and methods

2.1. Bacterial strains and media

LAB were isolated from pickled white cabbage (*Brassica oleracea* var. *capitata*), papaya (*Carica papaya*) and tomatoes (*Lycopersicon esculentum*) grown in Reunion Island and isolates were stored at −80 °C. Isolation was performed as previously described (Fessard et al., 2016). Briefly, from enumeration plates, different colonies were streaked out to obtain pure cultures. All isolates shared the ability to grow on MRS agar with cycloheximide and were catalase negative. *Lactobacillus plantarum* DSM 2601 and DSM20174, *Leuconostoc pseudomesenteroides* DSM 20193 and DSM5625, *Weissella cibaria* DSM 14295 and DSM 15878, *Weissella confusa* DSM 20196, *Weissella koreensis* DSM 15830 were used as reference strains for all experiments. Strain 1102001 (*W. confusa*), isolated from green pea juice from CTCPA was added to the study.

2.2. Ability to produce exopolysaccharides (EPS)

2.2.1. EPS production from sucrose

Homopolysaccharide production test was performed on MRS sucrose agar ($40\,g{\cdot}L^{-1}$) as previously described (Fessard et al., 2016). Experiments were performed at 25 °C, 30 °C and 37 °C.

2.2.2. Sequencing of glycansucrase encoding genes

Primers targeting genes encoding enzymes involved in the synthesis of EPS, dextransucrase and levansucrase were used in this study. The primer sets dsrk39-For/dsrk39-Rev and WconDex-fw/WconDex-rev were used to detect dextransucrase from *W. cibaria* and *W. confusa* strains, respectively (Bounaix et al., 2010b; Malang et al., 2015). The primer sets gtf-fw/gtf-rev and LevV-fw/LevV-rev were used to detect glucansucrase and levansucrase respectively, from *Leuconostoc* strains (Palomba et al., 2012). The PCR reaction was performed in a 50 μL volume with 10 μL of DNA solution. PCR was performed using a Bio-rad S100 Thermal Cycler and were checked by 0.8% agarose gel electrophoresis. PCR products were sequenced by Sanger method with dsrk39-For, WconDex-fw and gtf-fw primers as previously described (Fessard et al., 2016). The primer set FTF2-F/FTF2-R was also used to detect fructansucrase from *Weissella* and *Leuconostoc* isolates (Bounaix et al., 2009). The obtained sequences were compared with NCBI Nucleotide database using BLASTN program.

2.3. Identification of species and typing

DNA extraction was performed using Instagen™ protocol (Instagen Matrix, BIORAD, Marnes la Coquette, France). The supernatant containing DNA was stored at −20 °C until use.

2.3.1. Identification

All isolates were identified by 16S rRNA coding region sequencing. In case of uncertainty about the species, *pheS* gene sequencing was applied for *Leuconostoc*, *Lactococcus*, *Weissella* and *Fructobacillus* spp. isolates whereas *recA* gene sequencing was used to identify *Lactobacillus* spp. isolates, as previously described (Fessard et al., 2017). PCR products were sequenced by Sanger method with FD1m, pheS-21F, PlanF and ParaF primers as previously described (Fessard et al., 2016).

2.4. Genotyping

A rep-PCR method based on $(GTG)_5$ primer was used for intra-species genotypic discrimination. PCR was performed according to Versalovic et al. (1994) as previously described (Fessard et al., 2016). After electrophoresis, agarose gels were stained with ethidium bromide and the images were acquired with GelDoc (Biorad, Marnes la Coquette, France). The patterns were analysed with CLIQS 1D Pro (Core Laboratory Image Quantification Software, TotalLab, Newcastle upon Tyne, England), which considers both the presence/absence of bands and their relative intensity. The dendrogram was generated using Pearson coefficient correlation and the arithmetic average clustering algorithm (UPGMA). A coefficient of 0.15 was used to delimitate clusters. This coefficient was set up from triplicate electrophoresis of three independent experiments to take into account the experimental variability generated by the analyses (PCR-electrophoresis-image analysis).

2.5. Tolerance to acid, oxidative and osmotic stress and bile salts

Tolerance to stress was performed in sterile 96-well microplates. Bacterial isolates were grown for 48 h at 30 °C in MRS broth and 20 μL of this bacterial suspension were inoculated into 180 μL of corresponding broth. The control condition was MRS broth (pH 6.5). For acid stress, MRS broth acidified to pH 3.0 or pH 4.5 with $2\,mol{\cdot}L^{-1}$ HCl was used. MRS broth containing 5% or 8% NaCl was used for osmotic stress. And for oxidative stress, MRS broth containing 0.025% or 0.05% or 0.075% or 0.1% H_2O_2 was used. For tolerance to bile salts, MRS broth was supplemented with 0.1%, 0.2% or 0.3% of bile salts (Sigma Aldrich). Optical density (OD) at 660 nm was measured (Infinite M200 Pro, Tecan, Lyon, France) just after inoculation (OD_0) and after 24 h (OD_{24}) or 48 h (OD_{48}) of incubation at 37 °C. For acid, osmotic and oxidative stress, results were expressed as log (OD_{48}/OD_0) and indicated as follows: + + + for a log (OD_{48}/OD_0) superior to 0.5 considered as a high growth yield; + + for a log (OD_{48}/OD_0) comprised between 0.3 and 0.5 considered as a moderate growth; + for a log (OD_{48}/OD_0) comprised between 0.1 and 0.3 considered as a low growth; +/− for a log (OD_{48}/OD_0) comprised between 0.08 and 0.1 considered as a very poor growth; − for a log (OD_{48}/OD_0) inferior to 0.08 and was considered as no growth. For bile salts condition, results were expressed as a percentage of control (100% corresponding to OD_{24} in MRS broth without bile salts). Experiments were performed in three independent experiments analysed in triplicate.

2.6. Growth parameters

Determination of growth parameters was performed in sterile 96-well microplates. Bacterial isolates were grown for 48 h at 30 °C. A volume of 20 μL of this bacterial suspension was inoculated into 180 μL of MRS broth. Microplates were incubated at 6 different temperatures:

12 °C, 18 °C, 25 °C, 30 °C, 37 °C and 42 °C. OD at 660 nm was measured every 2 h (Infinite M200 Pro, Tecan, Lyon, France). Experiments were performed in three independent experiments analysed in triplicates. The maximum growth rate (μ_{max}) value was deduced from the curve ln (OD 600 nm) = f(time) using a primary growth model fitting from Sym'Previus software (http://symprevius.eu/en/). Optimum, minimal and maximal growth temperature (T_{opt}, T_{min} and T_{max}) and optimum growth rate (μ_{opt}) were deduced from a secondary growth model fitting also from Sym'Previus software (Rosso et al., 1993). The secondary growth model is based on the gamma concept and the model was used to fit the μ_{max} data as a function of temperature. The R^2 value estimates the goodness of fit of the model: the closest it is to 1, the highest is the fitting of the model to experimental values. Standard deviation was calculated to describe the spread of values towards the model mean. In order to confirm the cardinal temperature values predicted with Sym'Previus software, growth of isolates was also checked after 21 days of incubation at 2 °C, 4 °C, 6 °C, 8 °C, 10 °C, 12 °C, 15 °C, 42 °C or 45 °C as previously described.

2.7. Statistical analysis

The software XLSTAT (Addinsoft, Paris, France) was used for all statistical analyses. Significant differences versus a control or by pairs were tested with Dunnett's or Ryan, Einot, Gabriel, Welch q (REGWQ) tests respectively. A confidence interval of 95% was chosen for all statistical tests.

3. Results

3.1. Isolation and identification

A total of 77 LAB were isolated: 24 from papayas (6 different fruits), 47 from sliced cabbage (5 different samples) and 6 from tomatoes (1 fruit). The highest LAB population was observed for pickled cabbage samples with 8.4 log CFU·g^{-1}. LAB populations of tomato and papaya ranged between 2.9 and 5.1 log CFU·g^{-1}. Isolates were first identified by *16S* rRNA gene sequencing but distinction remained uncertain between several species. For further identification, *pheS* and *recA* gene sequencing was used respectively for *Weissella* and *Leuconostoc* species and for *Lactobacillus* isolates.

Sequencing showed the presence of 13 different species (number of isolates): *Lb. plantarum* (3), *Lb. paraplantarum* (2), *Lb. paralimentarius/kimchii* (1), *Fructobacillus tropaeoli* (1), *Lactococcus lactis* subsp. *lactis* (13), *Lc. pseudomesenteroides* (25), *Lc. citreum* (14), *Lc. mesenteroides* (7), *Lc. lactis* (1), *Weissella cibaria* (4), *W. confusa* (4), *W. paramesenteroides* (1) and *W. soli* (1) (Table 1). Species with a single isolate, *Fb. tropaeoli* 77 and *W. paramesenteroides* 37 on one side, and *Lb. paralimentarius/kimchi* 71, *Lc. lactis* 24 and *W. soli* 58 on the other side, were isolated from papaya and pickled cabbage respectively.

For species with multiple isolates, several isolation origins were observed. On the other side, for a given sample, several isolates from the same species could be obtained. Eventually, there was no correlation between a given species and an isolation material.

3.2. $(GTG)_5$-fingerprinting

All 77 isolates, all reference strains and strain 1102001 were subjected to rep-PCR. $(GTG)_5$ primer generated different patterns which were used for classification into clusters (Table 1 and Fig. 1). $(GTG)_5$ fingerprinting revealed a high diversity of genetic profiles since the 87 isolates or strains were clustered in 48 groups, using a threshold of 0.15. This threshold was set up from triplicate experiments to take into account experimental reproducibility.

The eight *Lactobacillus* spp. were classified into four $(GTG)_5$ groups. The two reference *Lb. plantarum* strains DSM2601 and DSM20174 were clustered in the same $(GTG)_5$ group. None of our isolates were classified in this group. Isolate 71 identified as *Lb. paralimentarius/kimchi* showed a specific profile. *Lb. paraplantarum* isolates (73 and 74) were clustered in the same $(GTG)_5$ group than two *Lb. plantarum* isolates (17a and 29a).

The 13 *L. lactis* subsp. *lactis* isolates were allocated to six distinct $(GTG)_5$ group, whereas most of them were isolated from sliced cabbage. Noteworthy, some isolates (6, 14, 19, 40, 41, 53 and 65) from different samples of papaya and sliced cabbage were grouped into the same cluster.

The 50 *Leuconostoc* spp. were allocated to 23 $(GTG)_5$ groups. *Lc. pseudomesenteroides* (27 strains), *Lc. mesenteroides* (7 strains) and *Lc. citreum* (15 strains) were spread in 12, 6 and 4 groups, respectively. None of the *Lc. pseudomesenteroides* isolates were grouped with the reference strains DSM20193 and DSM5625. However, similarity was observed between *Lc. pseudomesenteroides* isolates 12b, 27b and *W. koreensis* DSM15830. Two *Lc. citreum* isolates (33 and 13a) were grouped with the *Lc. citreum* reference strain DSM20188. *Lc. lactis* isolate 24 showed a genetic profile distinct from other *Leuconostoc* spp.

A high diversity was observed for the 15 *Weissella* spp. which were spread into 14 clusters. *W. cibaria* 64, 21, 30, DSM14295 and DSM15878 patterns presented some similarity but were not classified in the same cluster. $(GTG)_5$ profile of isolate 10b was clearly different from those of other *W. cibaria* isolates. *W. confusa* 16 and 17 presented the same profile and showed some similarity with *W. confusa* 59 and DSM20196 but were not classified in the same cluster. Profiles of *W. confusa* 1102001 and 38 were clearly different. Distinct profiles were observed also for *W. soli* 58 and *W. paramesenteroides* 37.

3.3. EPS production and glycansucrase gene detection

Neither of the *Lactobacillus* spp. isolates, nor the *L. lactis* isolates, nor *Fb. tropaeoli* 77, *W. paramesenteroides* 37 and *W. soli* 58 did produce EPS on sucrose medium (Table 1). On the contrary, all *Leuconostoc* isolates and all the isolates of other *Weissella* species produced EPS from sucrose and colony aspect was strain dependent, either liquid or creamy (Tables 1 and 2).

For these isolates, EPS production from sucrose was observed at 25 °C and 30 °C. At 37 °C, some isolates (60, 24, 1, 5, 6a, 28, 10b, 30, 38 and 1102001) did not produce EPS, whereas for some other isolates (DSM20193, DSM5625, 79, 33, 2, 16, 17 and 59) a change of the EPS phenotype was noticed (Table 2). The screening for potential glucansucrase genes from *Leuconostoc* spp. revealed 6 positive strains: *Lc. mesenteroides* 1, 5, 6a, 28 and *Lc. citreum* 2, 9a. *W. cibaria* and *W. confusa* isolates gave the expected fragment for the amplification of partial dextransucrase gene. Sequencing of the PCR products confirmed the similarity to glucansucrase or dextransucrase genes from databases (Table 2). Amplification with LevV-fw/LevV-rev and with FTF2-F/FTF2-R primers was not positive suggesting the absence of levansucrase and fructansucrase.

3.4. Growth yield in control MRS condition and tolerance to stress

Isolates of *Weissella*, *Leuconostoc* and *Fb. tropaeoli* from distinct $(GTG)_5$ group were chosen for further characterization regarding their tolerance to stress, to bile salts and their growth at different temperatures. Results were compared with reference strains and *Lactobacillus* isolates.

Lb. plantarum 75, *W. cibaria* 64 and 30, *W. confusa* 1102001 harboured the highest growth yields over 48 h compared to other isolates ($p < 0.0001$), with log OD_{48}/OD_0 values of 1.16 ± 0.00, 0.91 ± 0.08, 0.86 ± 0.16 and 0.88 ± 0.11, respectively. On the contrary, *W. paramesenteroides* 37 showed a low growth yield in MRS pH 6.5 37 °C compared to other isolates ($p < 0.0001$) (Table 3).

At a low initial pH of 4.5 (Table 3), log OD_{48}/OD_0 values were comprised between 0.04 (*Lc. pseudomesenteroides* 60) and 1.05 (*Lb. plantarum* 75). At a lower pH of 3, they were comprised between 0.03

Table 1
Species, origin, EPS production and rep-PCR groups of the isolates. EPS production is indicated as follows: (+) positive phenotype and (−) no production.

Genus	$(GTG)_5$ group	Species	Isolate (origin[a,b,c])	EPS production
Fructobacillus	Fb35	*Fb. tropaeoli*	77 (P[a]6)	−
Lactobacillus	Lb1	*Lb. paralimentarius/kimchii*	71 (C[b]7)	−
	Lb43	*Lb. paraplantarum*	73, 74 (C7)	−
	Lb43	*Lb. plantarum*	17a (P), 29a (T[c])	−
	Lb44	*Lb. plantarum*	75 (C7)	−
	Lb45	*Lb. plantarum*	DSM2601, DSM20174 (pickled cabbage)	−
Lactococcus	L32	*L. lactis*	6 (C2), 14, 19 (C4), 40, 41 (P5), 53 (C6), 65 (C7)	−
	L9	*L. lactis*	62 (C7)	−
	L26	*L. lactis*	20 (C4)	−
	L2	*L. lactis*	67 (C7)	−
	L28	*L. lactis*	70 (C7)	−
	ND	*L. lactis*	42 (P5)	−
	L31	*L. lactis*	82 (P6)	−
Leuconostoc	Lc25	*Lc. pseudomesenteroides*	DSM5625 (commercial starter)	+
	Lc24	*Lc. pseudomesenteroides*	57 (C6)	+
	Lc23	*Lc. pseudomesenteroides*	66 (C6)	+
	Lc27	*Lc. pseudomesenteroides*	89 (C7)	+
	Lc29	*Lc. pseudomesenteroides*	12 (P2)	+
	Lc22	*Lc. pseudomesenteroides*	48, 49, 50, 51, 52, 54, 55, 56 (C6), 61, 63, 68 (C7)	+
	Lc21	*Lc. pseudomesenteroides*	DSM20193 (sugar cane juice)	+
	Lc48	*Lc. pseudomesenteroides*	12b (T), 27b (P)	+
	Lc34	*Lc. pseudomesenteroides*	60 (C6)	+
	Lc6	*Lc. pseudomesenteroides*	79 (P6)	+
	Lc7	*Lc. pseudomesenteroides*	39 (P5)	+
	Lc8	*Lc. pseudomesenteroides*	11 (P2), 78, 81, 86, 87 (P6)	+
	Lc18	*Lc. lactis*	24 (C5)	+
	Lc14	*Lc. mesenteroides*	5a (P), 80 (P6)	+
	Lc12	*Lc. mesenteroides*	1 (P1)	+
	Lc10	*Lc. mesenteroides*	5 (C2)	+
	Lc13	*Lc. mesenteroides*	6a (P)	+
	Lc30	*Lc. mesenteroides*	28 (C5)	+
	Lc11	*Lc. mesenteroides*	47 (C6)	+
	Lc36	*Lc. citreum*	8 (P2), 23, 32 (C5)	+
	Lc37	*Lc. citreum*	2 (C2), 9 (P2), 29, 31 (C5), 72, 76 (C7), 84, 88 (P6)	+
	Lc4	*Lc. citreum*	9a (T)	+
	Lc5	*Lc. citreum*	33 (C5), 13a (T), DSM20188 (ND[d])	+
Weissella	W38	*W. cibaria*	64 (C7)	+
	W39	*W. cibaria*	21 (C4)	+
	W40	*W. cibaria*	DSM15878 (chili bo)	+
	W41	*W. cibaria*	DSM14295 (kimchi)	+
	W42	*W. cibaria*	30 (C5)	+
	W33	*W. cibaria*	10b (T)	+
	W20	*W. confusa*	1102001 (green pea juice)	+
	W15	*W. confusa*	16, 17 (C4)	+
	W16	*W. confusa*	DSM20196 (cane sugar)	+
	W17	*W. confusa*	59 (C6)	+
	W3	*W. confusa*	38 (P5)	+
	W47	*W. koreensis*	DSM15830 (kimchi)	+
	W46	*W. paramesenteroides*	37 (P4)	−
	W19	*W. soli*	58 (C6)	−

[a] P: papaya.

[b] C: cabbage.

[c] T: tomato.

[d] ND: not determined.

(*Lc. pseudomesenteroides* 56) and 0.22 (*W. confusa* 1102001). *Lb. plantarum* 17a and *W. cibaria* 10b were not affected by exposure to pH 4.5, as no significant differences were detected compared to control condition. Some isolates (73, 75, DSM2601, DSM20193, DSM5625, 39, 78, DSM20188, 21, 30, 64 and 1102001) showed a high growth yield in MRS pH 4.5, but they were clearly affected by this condition compared to control ($p < 0.0001$). At an initial pH of 3, the growth yield of all isolates was reduced compared to control condition ($p < 0.0001$) and most of isolates did not succeed to grow in this condition (Table 3). The most acid-tolerant isolates were: *Lb. paraplantarum* 73; *Lb. plantarum* 75; *Lc. pseudomesenteroides* 12b, 27b, DSM5625, DSM20193, 39; *Lc. lactis* 24; *Lc. mesenteroides* 28; *Lc. citreum* 33, 9a; *W. cibaria* 30, 64, and *W. confusa* DSM20196, 59 and 1102001.

In moderate saline stress (NaCl 5%), log OD_{48}/OD_0 values were comprised between 0.04 (*Lc. mesenteroides* 5) and 1.16 (*Lb. plantarum* 75). *Lb. plantarum* 75, 17a, *Lb. paraplantarum* 73, *W. cibaria* DSM15878 and *W. soli* 58 were the most tolerant to NaCl 5%, and no significant differences were detected compared to control condition (Table 3). *Lc. citreum* 33, *W. confusa* 38 and *W. paramesenteroides* 37 were only moderately affected by NaCl 5% ($p < 0.05$). Some isolates exhibited high growth in NaCl 5% (1102001, 16, 17, 60, 64, 30 and 59), but compared to control condition, growth was significantly reduced ($p < 0.0001$). When a stronger saline stress was applied (NaCl 8%), log OD_{48}/OD_0 values were comprised between 0.00 (*W. cibaria* 10b) and 0.97 (*Lb. plantarum* 75). *W. confusa* 38 seemed slightly affected by this condition but the difference with control condition was not significant. *W. soli* 58 was not significantly affected by exposure to NaCl 8%. Isolates 75 and 60 revealed a high growth yield in the presence of 8% NaCl, however they were sensitive to this condition ($p < 0.001$ and $p = 0.007$, respectively). The growth of all other isolates was severely

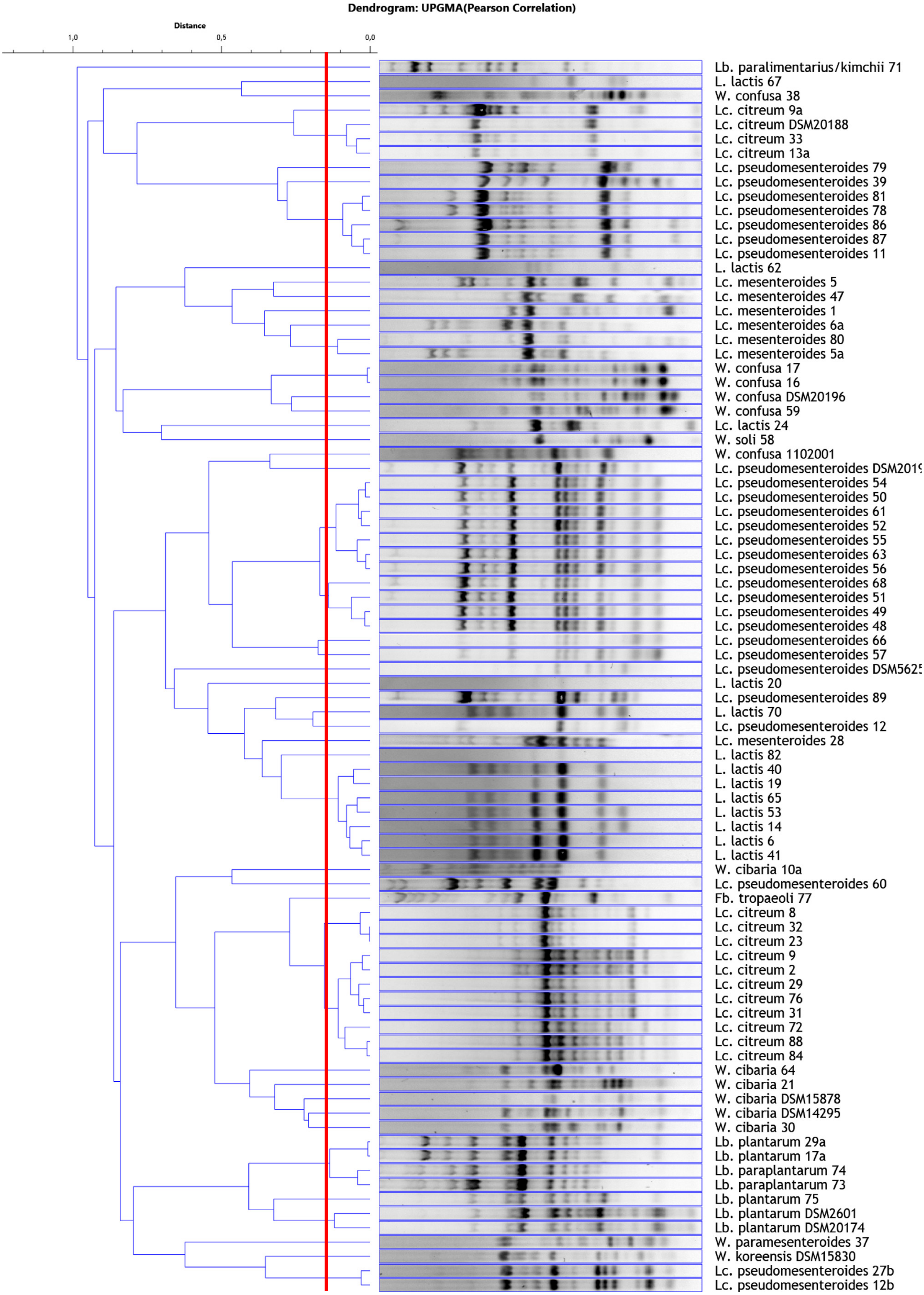

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Fig. 1. Dendrogram obtained from $(GTG)_5$-typing. The dendrogram was generated using Pearson coefficient correlation and the arithmetic average clustering algorithm (UPGMA). The vertical red line indicates the optimization coefficient of 0.15 used to delimitate clusters. A total of 48 genetic groups were generated for all isolates and strains. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

affected.

In a slight oxidative condition, log OD_{48}/OD_0 were comprised between 0.00 (5, DSM2601, 2, 79) and 1.15 (75). *Fb. tropaeoli* 77, *Lb. plantarum* 75, 17a, *Lc. pseudomesenteroides* DSM20193, DSM5625, 60, 39, *Lc. citreum* 9a, *W. cibaria* 21, 30, 64, DSM14295, DSM15878 and *W. confusa* 1102001 were not affected by exposure to 0.025% in H_2O_2 (Table 3). This concentration partially inhibited the growth of *W. confusa* 16 and 59 ($p < 0.05$). The growth of all other isolates was reduced in this condition. With 0.05% H_2O_2, only four isolates were not affected: *Fb. tropaeoli* 77, *Lc. pseudomesenteroides* 60 and *W. cibaria* 30 and 64. No significant differences were detected between 0.075% and 0.1% H_2O_2 conditions. This concentration in H_2O_2 was very efficient to impair growth of almost all LAB isolates. Only *Lc. pseudomesenteroides* 60 was not affected by 0.1% H_2O_2. *Fb. tropaeoli* 77 was also able to grow in this condition but compared to control condition its growth was significantly reduced ($p < 0.001$).

3.5. Tolerance to bile salts

None of the LAB strains was able to grow in 0.2% or 0.3% of bile salts after 24 h (data not shown). Tolerance to bile salts was thus performed with 0.1% over 24 h and expressed as a percentage of OD of control (MRS broth without bile salts). Four isolates showed the highest resistance to bile salts: *Lc. pseudomesenteroides* 12b, *W. paramesenteroides* 37, *Lc. citreum* DSM20188 and *Lb. plantarum* 75, with a percentage of growth comprised between 61% and 83.8% of that observed in control condition (Fig. 3). Tolerance of strain 12b was significantly higher than that of other isolates ($p < 0.001$), except compared to strains 37 and DSM20188. Other *Leuconostoc* strains showed a relatively good tolerance, with a percentage of growth comprised between 35% and 55.8% of control. *Weissella* isolates and *Lc. pseudomesenteroides* 60 were the less tolerant to bile salts, with a percentage of growth comprised between 14.9% and 32.6% of control. The lowest tolerance was observed for *Lc. pseudomesenteroides* 60 and *W. confusa* strains 38, 16, DSM20196 and 17. *Leuconostoc* strains 58, 89, 27b, 2, 39, 56, 79, 33, 78 and *Weissella* strains 58 and DSM15830 were not able to grow at 37 °C in 24 h in the presence of 0.1% bile salts: for these isolates, we cannot conclude regarding their tolerance to bile salts.

3.6. Temperature effect on growth rate

Cardinal temperatures and μ_{opt} were determined using Sym'Previus software by plotting μ_{max} as a function of temperature (12 °C, 18 °C, 25 °C, 30 °C, 37 °C, 42 °C) (Fig. 2). Predicted values obtained from Sym'Previus model and possible growth after incubation for 21 days are indicated in Table 4.

Optimal growth temperature T_{opt} of LAB isolates ranged between 25.1 °C (*Lc. citreum* 33) and 39.0 °C (*W. confusa* 59). The majority of *Leuconostoc* spp. harboured T_{opt} comprised between 25.0 and 30.0 °C. *Weissella* spp. and *Lactobacillus* spp. showed T_{opt} over 30 °C, above 34 °C for *Weissella* isolates 30, 16, 17, 59 and 58. LAB isolates harboured μ_{opt} values comprised between 0.107 h^{-1} (*Lc. pseudomesenteroides* 27b) and 0.998 h^{-1} (*W. confusa* 17) (Table 4). *W. confusa* isolates (38, 1102001,

Table 2
Influence of the temperature on EPS production from sucrose and glycansucrase gene detection.

Isolate	EPS production and colony aspect			Gene detection		
	25 °C	30 °C	37 °C	Glucansucrase sequence length (pb)	% identity	Species
Lc. pseudomesenteroides 12b	+ (creamy)	+ (creamy)	+/−	ND	ND	ND
Lc. pseudomesenteroides 27b	+ (creamy)	+ (creamy)	+/−	ND	ND	ND
Lc. pseudomesenteroides 56	+ (creamy)	+ (creamy)	+ (creamy)	ND	ND	ND
Lc. pseudomesenteroides 89	+ (liquid)	+ (creamy)	+ (creamy)	ND	ND	ND
Lc. pseudomesenteroides DSM20193	+ (liquid)	+ (liquid)	+ (creamy)	ND	ND	ND
Lc. pseudomesenteroides DSM5625	+ (liquid)	+ (liquid)	+ (creamy)	ND	ND	ND
Lc. pseudomesenteroides 60	+ (creamy)	+(creamy)	–	ND	ND	ND
Lc. pseudomesenteroides 39	+ (creamy)	+ (creamy)	+ (creamy)	ND	ND	ND
Lc. pseudomesenteroides 78	+ (creamy)	+ (creamy)	+ (creamy)	ND	ND	ND
Lc. pseudomesenteroides 79	+ (liquid)	+ (liquid)	+ (creamy)	ND	ND	ND
Lc. lactis 24	+ (creamy)	+ (creamy)	–	ND	ND	ND
Lc. mesenteroides 1	+ (liquid)	+ (liquid)	–	609	99% AP017935.1	*Lc. mesenteroides*
Lc. mesenteroides 5	+ (liquid)	+ (liquid)	–	613	99% DQ249318.1	*Lc. mesenteroides*
Lc. mesenteroides 6a	+ (liquid)	+ (liquid)	–	526	90% JQ619633.1	*Lc. mesenteroides*
Lc. mesenteroides 28	+ (liquid)	+ (creamy)	–	118	92% MG869733.1	*Lc. mesenteroides*
Lc. citreum 33	+ (creamy)	+ (creamy)	+/− (liquid)	ND	ND	ND
Lc. citreum DSM20188	+ (liquid)	+ (liquid)	+/−	ND	ND	ND
Lc. citreum 2	+ (liquid)	+ (liquid)	+ (creamy)	615	99% DQ873511.1	*Lc. citreum*
Lc. citreum 9a	+ (creamy)	+ (creamy)	+/−	606	99% DQ873511.1	*Lc. citreum*
W. cibaria 10b	+ (creamy)	+ (creamy)	–	828	99% GU237484.3	*W. cibaria*
W. cibaria 21	+ (creamy)	+ (creamy)	+/−	823	99% GU237484.3	*W. cibaria*
W. cibaria 30	+ (creamy)	+ (liquid)	–	916	98% GU237484.3	*W. cibaria*
W. cibaria DSM14295	+ (creamy)	+ (creamy)	+/−	825	99% HE818409.1	*W. cibaria*
W. cibaria DSM15878	+ (creamy)	+ (creamy)	+ (granular)	885	99% GU237484.3	*W. cibaria*
W. cibaria 64	+ (creamy)	+ (creamy)	+/−	657	99% GU237484.3	*W. cibaria*
W. confusa 16	+ (creamy)	+ (creamy)	+ (granular)	210	98% KP729387.1	*W. confusa*
W. confusa 17	+ (creamy)	+ (creamy)	+ (granular)	234	98% KP729387.1	*W. confusa*
W. confusa DSM20196	+ (creamy)	+ (creamy)	+/−	29	100% KP729387.1	*W. confusa*
W. confusa 59	+ (creamy)	+ (creamy)	+ (granular)	182	99% KP729387.1	*W. confusa*
W. confusa 38	+ (creamy)	+ (creamy)	–	39	97% KP729387.1	*W. confusa*
W. confusa 1102001	+ (creamy)	+ (creamy)	–	54	91% KP729387.1	*W. confusa*

+: observed EPS production on MRS sucrose; +/−: weak EPS production, −: no EPS production; ND: no amplification.

Table 3
Tolerance of isolates to pH, to sodium chloride and to hydrogen peroxide. Results are indicated as follows: (+ + +) high growth, (+ +) moderate growth, (+) poor growth, (+/−) very poor growth and (−) no growth. Significant differences are calculated from Log OD_{48}/OD_0 values in acidic or osmotic or oxidative conditions and indicated as follows: $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*} < 0.05$ from Dunnett's test versus control condition (pH 6.5). No indication of significant degree means no significant difference (confidence of 95%).

Isolate	Control condition	pH 4.5	pH 3	NaCl 5%	NaCl 8%	0,025% H_2O_2	0,05% H_2O_2	0,1% H_2O_2
Fructobacillus								
77	+ +	$+^{***}$	$-^{***}$	$+^{***}$	$+^{***}$	+ +	+ +	$++^{***}$
Lactobacillus								
73	+ + +	$++^{***}$	$+/-^{***}$	+ + +	$-^{***}$	$-^{***}$	$-^{***}$	$-^{***}$
75	+ + +	$+++^{***}$	$+^{***}$	+ + +	$+++^{***}$	+ + +	$-^{***}$	$-^{***}$
17a	+ + +	+ + +	$-^{***}$	+ + +	$+^{***}$	+ + +	$-^{***}$	$-^{***}$
DSM2601	+ + +	$++^{***}$	$-^{***}$	$-^{***}$	$-^{***}$	$-^{***}$	$-^{***}$	$-^{***}$
Leuconostoc								
12b	+ + +	$+^{***}$	$+^{***}$	$+^{***}$	$+/-^{***}$	$-^{***}$	$-^{***}$	$-^{***}$
27b	+ + +	$+^{***}$	$+^{***}$	$+/-^{***}$	$+/-^{***}$	$-^{***}$	$-^{***}$	$-^{***}$
56	+ + +	$+^{***}$	$-^{***}$	$+^{***}$	$-^{***}$	$-^{***}$	$-^{***}$	$-^{***}$
89	+ +	$-^{***}$	$-^{***}$	$+^{***}$	$-^{***}$	$-^{***}$	$-^{***}$	$-^{***}$
DSM20193	+ + +	$++^{***}$	$+^{***}$	$++^{***}$	$-^{***}$	+ + +	$++^{***}$	$-^{***}$
DSM5625	+ + +	$++^{*}$	$+^{***}$	$++^{**}$	$-^{***}$	+ + +	$++^{**}$	$-^{***}$
60	+ +	$-^{***}$	$-^{***}$	$+++^{**}$	$+++^{**}$	+ +	+ +	+ +
39	+ + +	$++^{***}$	$+^{***}$	$++^{***}$	$+/-^{***}$	+ + +	$-^{***}$	$-^{***}$
78	+ + +	$+^{***}$	$-^{***}$	$+^{***}$	$-^{***}$	$+^{***}$	$-^{***}$	$-^{***}$
79	+ +	$-^{***}$	$-^{***}$	$-^{***}$	$-^{***}$	$-^{***}$	$-^{***}$	$-^{***}$
24	+ +	$+/-^{**}$	$+/-^{**}$	$+^{**}$	$-^{**}$	$-^{***}$	$-^{***}$	$-^{***}$
1	+ +	$+/-^{***}$	$-^{***}$	$+/-^{***}$	$-^{***}$	$-^{***}$	$-^{***}$	$-^{***}$
5	+ + +	$+/-^{***}$	$-^{***}$	$-^{***}$	$-^{***}$	$-^{***}$	$-^{***}$	$-^{***}$
6a	+ +	$+/-^{***}$	$-^{***}$	$+^{***}$	$+^{***}$	$-^{***}$	$-^{***}$	$-^{***}$
28	+ +	$+/-^{***}$	$+/-^{***}$	$+^{***}$	$+^{***}$	$-^{***}$	$-^{***}$	$-^{***}$
33	+ + +	$+^{***}$	$+/-^{***}$	$++^{*}$	$+/-^{***}$	$++^{***}$	$-^{***}$	$-^{***}$
DSM20188	+ + +	$++^{***}$	$-^{***}$	$+^{***}$	$-^{***}$	$++^{***}$	$-^{***}$	$-^{***}$
2	+ +	$-^{***}$	$-^{***}$	$-^{***}$	$-^{***}$	$-^{***}$	$-^{***}$	$-^{***}$
9a	+ + +	$+^{***}$	$+^{***}$	$+^{***}$	$-^{***}$	+ + +	$+/-^{***}$	$-^{***}$
Weissella								
10b	+ +	+ +	$-^{***}$	$+^{**}$	$-^{***}$	$++^{**}$	$-^{***}$	$-^{***}$
21	+ + +	$++^{***}$	$-^{***}$	$++^{**}$	$+^{***}$	+ + +	$+++^{***}$	$-^{***}$
30	+ + +	$++^{***}$	$+/-^{***}$	$+++^{***}$	$+^{***}$	+ + +	+ + +	$+^{***}$
DSM14295	+ + +	$+^{***}$	$-^{***}$	$++^{***}$	$+^{***}$	+ + +	$-^{***}$	$-^{***}$
DSM15878	+ +	$-^{**}$	$-^{*}$	+ +	$-^{*}$	+ +	$-^{***}$	$-^{**}$
64	+ + +	$+++^{***}$	$+/-^{***}$	$+++^{***}$	$+^{***}$	+ + +	+ + +	$+^{***}$
16	+ + +	$+^{***}$	$-^{***}$	$+++^{***}$	$+/-^{***}$	$+++^{*}$	$-^{***}$	$-^{***}$
17	+ + +	$+^{***}$	$-^{***}$	$+++^{***}$	$-^{***}$	$+++^{***}$	$-^{***}$	$-^{***}$
DSM20196	+ + +	$+^{***}$	$+^{***}$	$+^{***}$	$+^{***}$	$+++^{***}$	$-^{***}$	$-^{***}$
59	+ + +	$+^{***}$	$+^{***}$	$+++^{***}$	$+^{***}$	$+++^{*}$	$-^{***}$	$-^{***}$
38	+ + +	$+/-^{***}$	$-^{***}$	$++^{*}$	+ +	$+^{***}$	$-^{***}$	$-^{***}$
DSM15830	+ + +	$+^{***}$	$-^{***}$	$-^{***}$	$-^{***}$	$-^{***}$	$-^{***}$	$-^{***}$
37	+	$+/-^{***}$	$-^{***}$	$+^{*}$	$+/-^{***}$	$-^{***}$	$-^{***}$	$-^{***}$
58	+ +	$-^{***}$	$-^{***}$	+ +	+ +	$-^{***}$	$-^{***}$	$-^{***}$
1102001	+ + +	$+++^{***}$	$+^{***}$	$+++^{***}$	$+^{***}$	+ + +	$-^{***}$	$-^{***}$

16, 59, 17) and *W. cibaria* isolates (64, 30, 10b) showed the highest μ_{opt} values. Generally, *Leuconostoc* isolates shown the lowest μ_{opt} values.

LAB isolates exhibited minimal growth temperatures T_{min} comprised between 0.6 °C (*W. confusa* 59) and 16.7 °C (*W. cibaria* DSM15878). *Leuconostoc* spp. harboured T_{min} comprised between 1.0 °C (*Lc. mesenteroides* 5) and 11.9 °C (*Lc. pseudomesenteroides* 79). *Weissella* spp. harboured T_{min} comprised between 0.6 °C (*W. confusa* 59) and 16.7 °C (*W. cibaria* DSM15878). *Fructobacillus* and *Lactobacillus* spp. harboured T_{min} comprised between 3.7 °C (*Fb. tropaeoli* 77) and 14.9 °C (*Lb. paraplantarum* 73). Generally, *Leuconostoc* spp. have shown the lowest T_{min} values. Some Sym'Previus T_{min} values were not confirmed by incubation test for 21 days, as for *Lc. pseudomesenteroides* 39, 89, DSM5625 and DSM20193 or *W. confusa* 16 and 17.

Maximal growth temperature T_{max} of LAB isolates ranged between 37.5 °C (*Lc. pseudomesenteroides* 78) and 49.3 °C (*W. confusa* 16). The majority of *Leuconostoc* spp. harboured T_{max} comprised between 37.0 °C and 43.0 °C which was confirmed by incubation for 21 days at 42 °C. Incubation for 21 days at 45 °C confirmed that only two *Leuconostoc* isolates were able to grow between 42 °C and 45 °C, as *Lc. lactis* 24 and *Lc. pseudomesenteroides* 60. *Weissella* spp. harboured T_{max} comprised between 38.1 °C (*W. soli* 58) and 49.3 °C (*W. confusa* 16). *Fructobacillus* and *Lactobacillus* spp. harboured T_{max} comprised between 40.0 °C (*Lb. plantarum* DSM2601) and 46.5 °C (*Lb. plantarum* 75). In most case, incubation for 21 days resulted in maximal growth temperatures which fell into the confident interval given by the model, except for some strains for which the model mostly proposed higher T_{max}.

4. Discussion

The aim of this paper was to collect and characterize LAB isolates from tropically grown fruits and vegetables for possible application in food industry, especially for fruits and vegetables.

In this study, genetic and phenotypic characterization of 77 autochthonous LAB isolated from papaya, tomato and sliced cabbage was performed. Among the species the most frequently detected, *Lc. mesenteroides* was commonly isolated from fresh fruits and vegetables, as raw prickly pear, sweet cherry and raw peppers (Di Cagno et al., 2016, 2011b, 2009a), *Lc. pseudomesenteroides* and *Lc. citreum* have been isolated from ripe mulberries, fresh tomato, fresh coffee cherries and banana fruit (Chen et al., 2017, 2010; Leong et al., 2014; Trias et al.,

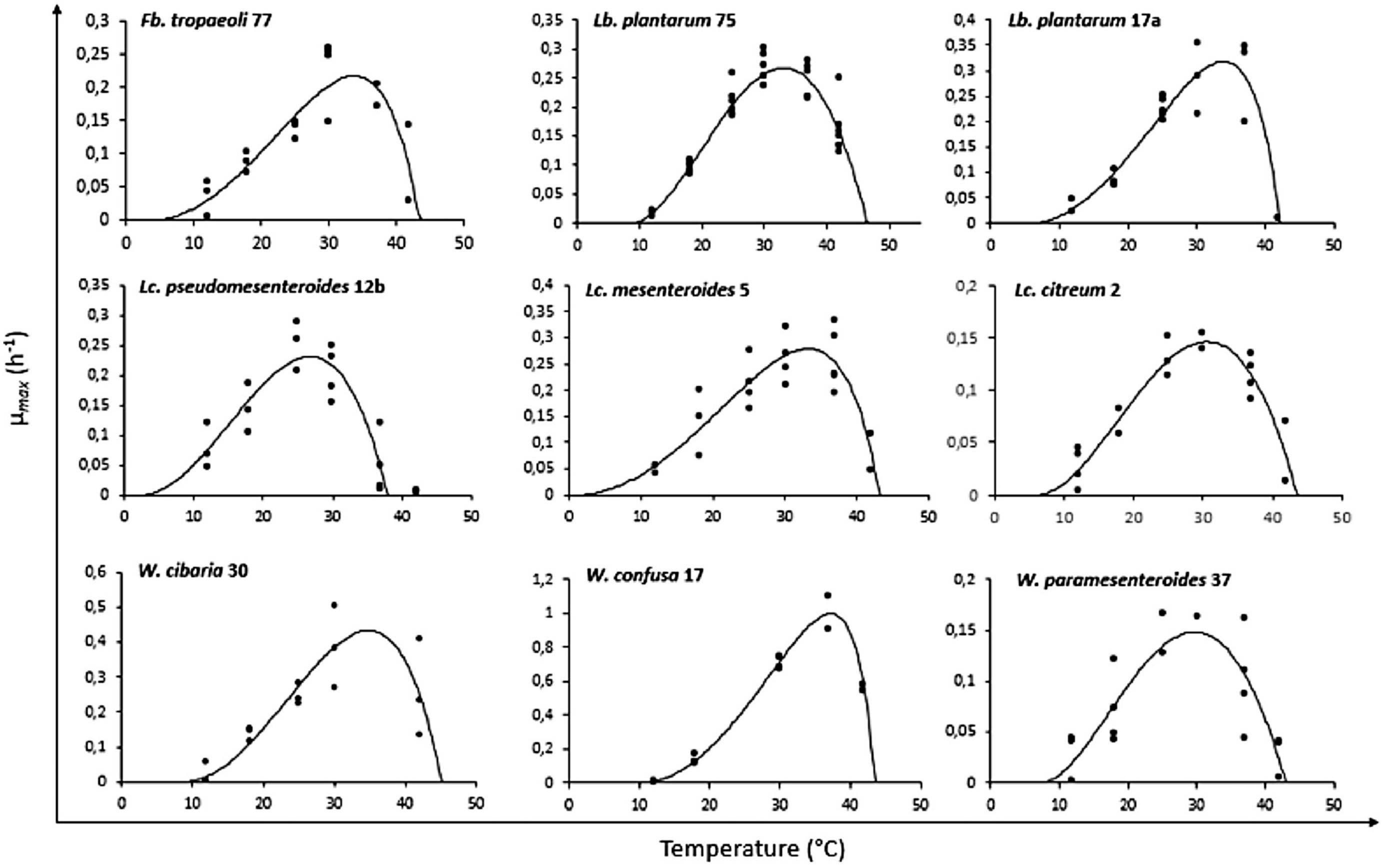

Fig. 2. Influence of temperature on the maximum growth rate μ_{max} (h^{-1}) for representative isolates. Curve lines are predicted model calculated by Sym'Previus for each isolate. Points are the μ_{max} values calculated by Sym'Previus from experimental growth curves.

2008). *Lc. lactis*, hereby isolated from cabbage, is mainly associated with dairy and vegetable-based fermented food, including kimchi (Chen et al., 2012; Cho et al., 2006; Vos et al., 2011). To the best of our knowledge, isolation of *Lc. lactis* from raw vegetable or ready-to-eat crude vegetable has not been reported. *W. cibaria*, *W. confusa*, *Lb. plantarum* and *Lb. paraplantarum* were the second-most-commonly detected species. These species are frequently isolated from fresh fruits and vegetables (Chen et al., 2010; Di Cagno et al., 2013, 2011a, 2009b; Emerenini et al., 2013; Trias et al., 2008). *Weissella* and *Lactobacillus* frequently occur in spontaneous fermentation of fruits or vegetables (Fessard and Remize, 2017), highlighting their natural adaptation to fruit and vegetable environments. Our study revealed also the presence of isolates of the species *W. soli* (cabbage), *W. paramesenteroides* (papaya) and *Fb. tropaeoli* (papaya). *W. paramesenteroides* has been isolated from a variety of fermented fruit and vegetable (Chen et al., 2013b, 2013a; Escalante-Minakata et al., 2008; Lan et al., 2009), and a single study reported its isolation from banana fruit (Chen et al., 2017). *W. soli* has been detected in silage fermentation of vegetable residues (cabbage, Chinese cabbage and lettuce) (Yang et al., 2010). *Fb. tropaeoli* was first isolated from a flower of *Tropaeolum majus* in South Africa (Endo et al., 2011) and was further isolated from spontaneous cocoa fermentation together with *W. fabalis* (Snauwaert et al., 2013). Only recently, *Fb. tropaeoli* has shown fruit origins (Franquès et al., 2017; Ruiz Rodríguez et al., 2017). Due to its frequent presence in raw milk, *L. lactis* has been extensively used as starter culture for dairy foods, contributing to the development of texture by producing exopolysaccharides (Casalta and Montel, 2008). In a recent study, two groups have been proposed for *L. lactis lactis* subspecies, “domesticated” and “environmental”. The latter appears to be the main contributor to genetic and phenotypic diversity within the subspecies (Laroute et al., 2017). The availability of new isolates from our study would be useful to understand the relationships between origin and phenotypic features. Besides, plant material is the main natural habitat of *L. lactis* and this species has been detected in fresh and frozen corn, corn silks, navy beans, cabbage, lettuce or peas (Vos et al., 2011), sprouted seeds and grapefruit juice (Kelly et al., 1996), by-products of pineapple and cherry pulp processing (Garcia et al., 2016), ripe mulberries (Chen et al., 2010) and fresh coffee cherries (Leong et al., 2014). Our results are then consistent with previous detection of these species from fruit and vegetable environments. It also revealed the presence of several species, like *Lc. lactis* or *W. paramesenteroides*, rarely isolated from fresh fruit and vegetables but rather from fermented foods.

Rep-PCR revealed a high diversity of genetic profiles. The 77 LAB isolates were clustered into 41 genetic groups, which is a high number of groups regarding the limited number of species and samples. The highest diversity was observed for *Leuconostoc* and *Weissella* isolates. Indeed, the 11 *Weissella* isolates were allocated to 10 genetic groups and were isolated from seven samples from three different raw material. From this observed genetic diversity, we hypothesized a phenotypic diversity which could be used as a stock for bacteria of technological interest.

Fermentation and biopreservation of minimally-processed foods from plant origin require selected strains with desirable properties. The use of LAB for this purpose presents many advantages regarding their history of use in foods and their ability to adapt to the specific conditions of these raw materials. The advantages of autochthonous strains rely of the assumption of a higher stability in their natural environment to compete microbial contaminants, and a better adaptative ability to their niche (Beganović et al., 2014; Di Cagno et al., 2008, 2009b; Fessard et al., 2016; de Souza and Dias, 2017). Although there is not a single protocol to select starter or bioprotective or probiotic strains and each application requires a tailored selection, several common traits are

Table 4

Optimum growth rate (μ_{opt}) and cardinal growth temperatures (T_{opt}, T_{min} and T_{max} respectively for optimum, minimal and maximal temperatures). Results represents the predicted values calculated by Sym'Previus software from three independent experiments. Mean ± standard deviation (SD) is shown. R^2 indicates the goodness of fit to the model. T_{min} and T_{max} observed in 21 days correspond to the minimal and maximal temperature, respectively, at which a significant growth was observed after 21 days of incubation.

Species	Isolate	μ_{opt} (h^{-1}) ± SD[a]	T_{opt} (°C) ± SD	T_{min} (°C) ± SD	T_{min} observed in 21 days	T_{max} (°C) ± SD	T_{max} observed in 21 days	R^2
Fb. tropaeoli	77	0.217 ± 0.047	33.8 ± 3.7	3.7 ± 6.6	10	43.4 ± 1.9	42	0.768
Lactobacillus	73	0.301 ± 0.038	32.9 ± 1.6	14.9 ± 2.5	6	45.7 ± 3.2	> 45	0.937
	75	0.268 ± 0.016	33.0 ± 0.8	8.3 ± 1.1	8	46.5 ± 1.4	42–45	0.954
	17a	0.318 ± 0.036	33.9 ± 1.7	5.5 ± 3.5	8	42.1 ± 0.2	42–45	0.92
	DSM 2601	0.298 ± 0.156	34.0 ± 2.4	9.8 ± 4.5	8	40.0 ± 12.8	42–45	0.834
Leuconostoc	12b	0.231 ± 0.040	26.7 ± 3.9	2.4 ± 10.5	2–4	37.9 ± 1.0	< 42	0.98
	27b	0.107 ± 0.042	28.7 ± 5.1	10.4 ± 5.8	6	42.6 ± 1.4	42	0.723
	56	0.263 ± 0.024	28.8 ± 1.1	10.8 ± 0.7	8	42.5 ± 0.2	< 42	0.998
	89	0.265 ± 0.016	29.9 ± 1.4	4.4 ± 2.1	8	42.5 ± 0.2	< 42	0.999
	DSM 20193	0.127 ± 0.031	30.6 ± 6.6	4.2 ± 2.7	8	42.7 ± 1.2	< 42	0.782
	DSM 5625	0.192 ± 0.031	31.0 ± 1.8	11.1 ± 1.6	8	38.3 ± 1.0	< 42	0.894
	60	0.186 ± 0.024	33.3 ± 2.4	4.3 ± 6.5	10	50.3 ± 7.8	42–45	0.979
	39	0.229 ± 0.052	27.5 ± 2.7	11.2 ± 4.3	6	43.1 ± 1.1	42	0.984
	78	0.205 ± 0.107	31.8 ± 6.4	9.0 ± 6.4	6	37.5 ± 1.6	42	0.787
	79	0.223 ± 0.047	27.6 ± 2.3	11.9 ± 1.1	8	42.9 ± 0.9	42	0.86
	24	0.288 ± 0.118	29.7 ± 5.4	10.5 ± 4.4	8	42.7 ± 1.7	42–45	0.895
	1	0.244 ± 0.049	29.6 ± 4.8	3.6 ± 13.5	< 2	45.6 ± 5.1	42	0.52
	5	0.279 ± 0.035	33.2 ± 2.5	1.0 ± 7.4	< 2	43.1 ± 1.0	< 42	0.787
	6a	0.202 ± 0.007	28.6 ± 0.6	6.6 ± 1.1	6	43.0 ± 0.2	< 42	0.999
	28	0.322 ± 0.062	26.1 ± 3.9	8.1 ± 13.2	8	42.2 ± 0.4	< 42	0.967
	33	0.231 ± 0.012	25.1 ± 1.5	7.1 ± ND	6	43.1 ± 0.4	< 42	0.995
	DSM 20188	0.261 ± 0.007	26.9 ± 0.5	3.5 ± 1.4	6	39.2 ± 0.3	> 42	0.999
	2	0.146 ± 0.024	30.5 ± 2.9	6.0 ± 4.7	2	43.6 ± 1.5	< 42	0.821
	9a	0.191 ± 0.047	31.8 ± 4.3	6.9 ± 5.5	8	42.9 ± 1.2	< 42	0.731
Weissella	10b	0.571 ± 0.144	30.9 ± 3.8	16.2 ± 8.0	8	44.4 ± 2.6	42–45	0.823
	21	0.209 ± 0.050	30.5 ± 4.1	8.7 ± 4.9	6	42.9 ± 1.3	42–45	0.794
	30	0.433 ± 0.120	34.8 ± 3.7	8.7 ± 4.9	6	45.1 ± 5.0	42–45	0.726
	DSM 14295	0.264 ± 0.031	29.8 ± 2.0	6.7 ± 4.8	8	43.1 ± 0.8	42–45	0.876
	DSM 15878	0.255 ± 0.052	31.0 ± 2.4	16.7 ± 2.1	6	42.9 ± 1.1	42–45	0.942
	64	0.387 ± 0.034	33.8 ± 1.3	6.7 ± 2.3	6	47.0 ± 2.7	42–45	0.981
	16	0.418 ± 0.066	32.1 ± 2.8	12.0 ± 0.8	10	49.3 ± 7.3	42–45	0.881
	17	0.998 ± 0.080	37.2 ± 0.9	10.1 ± 0.1	8	43.5 ± 0.8	42–45	0.993
	DSM 20196	0.199 ± 0.040	33.0 ± 4.5	1.8 ± 20.9	8	42.5 ± 0.9	42–45	0.886
	59	0.601 ± 0.185	39.0 ± 4.0	0.6 ± 6.5	6	43.1 ± 4.2	42–45	0.981
	38	0.336 ± 0.120	30.5 ± 4.3	8.4 ± 9.2	10	48.2 ± 8.8	42–45	0.599
	37	0.148 ± 0.035	29.7 ± 3.6	7.2 ± 6.0	8	43.0 ± 1.3	42–45	0.693
	58	0.264 ± 0.570	34.2 ± 10.8	16.2 ± 5.7	6	38.1 ± 13.7	42–45	0.749
	1102001	0.359 ± 0.087	31.0 ± 2.5	10.7 ± 2.5	10	47.8 ± 5.7	42–45	0.866

[a] SD: standard deviation.

considered whatever the context (Bevilacqua et al., 2012; Kostinek et al., 2005; Leroi et al., 2015). They are mainly related to adaptation of strains to technological conditions, such as acidic medium, salt addition or incubation temperature. Moreover, safety of strains is of crucial importance. Regarding probiotic selection, expectations of strain properties are different from those of fermentation starters or biocontrol agents. A step-by-step procedure has been proposed, investigating stress tolerance, adhesion ability, antipathogenic activity, safety assessment, host-associated functional properties, industrial requirements and omics characterization, before clinical trials (de Melo Pereira et al., 2018).

The behaviour of LAB at different temperatures and the determination of growth parameters are thus important to consider for a rational choice of strains for a specific application. Growth of *W. cibaria* and *W. confusa* isolates was detected between 6 °C and 45 °C, in accordance with those found in the literature (Björkroth et al., 2002; Fusco et al., 2015). *Leuconostoc* isolates had optimum growth between 25.1 °C and 33.3 °C, and growth up to 37 °C was strain dependent, which corresponds to the description of *Leuconostoc* spp. (Vos et al., 2011). *Lc. mesenteroides* 1 and 5, *Lc. citreum* 2 and *Lc. pseudomesenteroides* 12b have shown particular ability to grow at low temperatures, which it is a technological criteria of importance for their application in fruit and vegetable fermentation (Fessard and Remize, 2017). In our study, μ_{max} values of *Leuconostoc* isolates were lower than those observed for *Weissella* or *Lactobacillus* isolates. They were in the same range than those reported by Ricciardi et al. (2009) and Drosinos et al. (2006). Maximum growth rate is clearly species- and strain-dependent and several factors may affect this value such as temperature, pH, oxygen or presence of toxic compounds. Regarding growth parameters, temperature control appears here to be a potent lever to favour the growth of certain LAB isolates.

Low pH and high salt concentration are often used as selective conditions for LAB over food processing steps. Exposure to low pH affected the growth of all LAB strains, but *Weissella* and *Lactobacillus* spp. were more tolerant than *Leuconostoc* spp. Growth of *W. cibaria* strain in MRS broth adjusted to pH 3 has been reported by Patel et al. (2012). Interestingly, *Weissella* and *Lactobacillus* strains were also the most tolerant to salt stress, especially *W. soli* 58, *W. confusa* 38 and *Lb. plantarum* 75, which were able to grow in 8% NaCl, in accordance with Lee et al. (2012) and Papamanoli et al. (2003) which reported data for strains from the same species.

Unexpectedly, *Fb. tropaeoli* 77, *Lc. pseudomesenteroides* 60 and *W. cibaria* 21, 30 and 64 were the most tolerant to hydrogen peroxide, whereas *Lb. plantarum* strains have already been shown to be highly tolerant to exposure to 0.1% H_2O_2 for 30 min (Parente et al., 2010). Glutathione (GSH), a non-protein thiol compound, has been described in *Lactococcus* and *Lactobacillus* spp. and may play a role in the protection towards an oxidative stress (Zhang and Li, 2013). LAB are catalase negative but some strains may possess a manganese-dependent form.

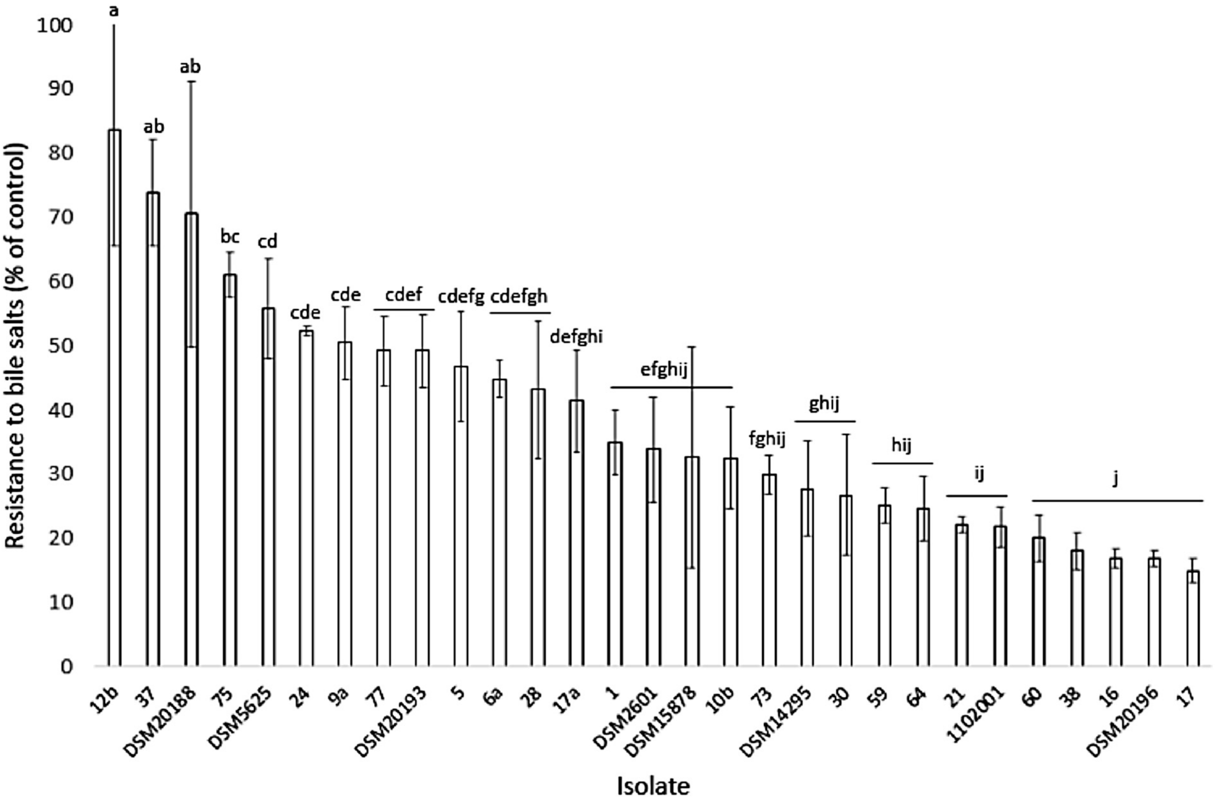

Fig. 3. Resistance of LAB isolates to 0.1% of bile salts (mean ± standard deviation). Results are expressed in percentage of OD600nm compared to control without bile salts. Different letters between isolates indicate significant differences with REGWQ test ($p < 0.05$).

Tolerance of LAB to bile salts has been associated with their capacity to metabolize the bile salts (van de Guchte et al., 2002) and constitute an important trait for the selection of cultures which can survive in gut. In our study, LAB isolates were able to grow only in 0.1% of bile salts which is quite low compared to the data reported in the literature. Concentrations of 0.15–0.3% of bile salts have been recommended as a suitable concentration for the selection of probiotic bacteria for human use (Boke et al., 2010). However, experimental time exposure to bile salts is generally limited to 6 h, which might explain the apparent discrepancy. Generally, *Leuconostoc* isolates have shown good tolerance to bile salts while *W. cibaria* and *W. confusa* isolates were the most sensitive. *Lc. pseudomesenteroides* 12b, *W. paramesenteroides* 37, and *Lb. plantarum* 75 isolates were the most tolerant to bile salts. If some *Lb. plantarum* strains were shown to be resistant to 2% bile salts (Papamanoli et al., 2003), tolerance to bile salts of *Lc. pseudomesenteroides* and *W. paramesenteroides* is not described.

Exopolysaccharides are important in the manufacture of dairy products and have gained interest recently for the manufacture of fruit or vegetable puree and smoothies (Di Cagno et al., 2011a; Juvonen et al., 2015). The screening for EPS production performed on sucrose medium revealed that only *Leuconostoc* spp., *W. cibaria* and *W. confusa* isolates produced EPS from sucrose. The literature qualified *W. cibaria*, *W. confusa* and *Leuconostoc* spp. as high producers of EPS (Di Cagno et al., 2016; Galle et al., 2010; Maina et al., 2008; Malang et al., 2015; Wolter et al., 2014), and no dextran was produced from sucrose by *Fb. tropaeoli* strain (Endo et al., 2011), which supports our results. However, the production of EPS was also previously reported for *Lb. plantarum* strains, isolated from sourdough and fish (Di Cagno et al., 2006; Hongpattarakere et al., 2012) and for *L. lactis* strain isolated from raw milk (Van der Meulen et al., 2007) but our isolates of these species did not produce EPS. The ability of LAB to produce EPS from sucrose is due to the action of one sucrase enzyme, either glucansucrase or fructansucrase (van Hijum et al., 2006). In our study, partial sequencing of glucansucrase genes was positive for *W. cibaria*, *W. confusa*, *Lc. mesenteroides* and *Lc. citreum* isolates. Several studies already reported glucan production or glucansucrase activity from *Lc. mesenteroides* and *Lc. citreum* strains (Bounaix et al., 2010a; Kang et al., 2014; Passerini et al., 2015; Song et al., 2016; Zannini et al., 2016) as well as for *W. cibaria* and *W. confusa* (Amari et al., 2013; Baruah et al., 2017; Bounaix et al., 2010b). Little is known about sucrase enzyme from *Lc. pseudomesenteroides* spp. and none of our *Lc. pseudomesenteroides* isolates producing EPS were positive for sucrase enzyme encoding genes. Dextran production constitutes a possible desirable technological trait for our isolates. Our study revealed also that temperatures comprised between 25 °C and 30 °C were optimum for the production of EPS. It has been shown that EPS production was higher at temperature comprised between 15 °C and 20 °C for *W. cibaria* (Hu and Gänzle, 2018). *W. confusa* dextransucrase activity was higher between 20 °C and 30 °C (Amari et al., 2013). These observations could explain the absence of EPS production at 37 °C observed for some isolates.

Our study provides a stock of autochthonous LAB species from fruits and vegetables with phenotypic characteristics useful for application in food. Altogether, *Weissella* strains, especially *W. cibaria* 64 and 30 were particularly tolerant to acidic, osmotic and oxidative conditions and produce EPS. However, until now, this species is not used as commercial starter (Fessard and Remize, 2017). *W. soli* 58 has shown high tolerance to osmotic conditions but was a relatively poor sugar fermenter in MRS broth. *W. paramesenteroides* 37 was one of the isolates the most tolerant to bile salts. The group of *Leuconostoc* isolates harbours a variety of diverse phenotypes. Among those, strain 12b showed a high tolerance to bile salts while strain 60 was particularly tolerant to osmotic and oxidative stress. *Lc. mesenteroides* 1 and 5 and *Lc. citreum* 2 were particularly tolerant to low temperatures. *Lb. plantarum* 75 was a high sugar fermenter, highly tolerant to low pH, salts and bile salts. For all those strains, no biogenic amine production from lysine, ornithine, tyrosine and histidine, and also the absence of detection of histidine, tyrosine and ornithine decarboxylase genes was observed (data not shown), but the possibility of biogenic amine production via the arginine deiminase pathway has to be checked.

W. cibaria 30 and 64, *Lc. pseudomesenteroides* 12b and 60 and *Lb. plantarum* 75 are powerful candidates for fruits and vegetables fermentation, whereas *W. soli* 58, *Fb. tropaeoli* 77, *Lc. mesenteroides* 1 and 5 could be investigated as preservative cultures for fruits and vegetables.

Despite their frequent detection in foods, genetic and phenotypic features of some LAB species remain poorly documented. LAB species mostly described are *L. lactis* and *Lb. plantarum* mainly due to their long and safe history of application, especially for dairy products (Leroy and De Vuyst, 2004). Our study provides a useful description of several autochthonous LAB isolates from other species often encountered in fruits and vegetables, including *Lactobacillus*, *Leuconostoc*, *Weissella* and *Fructobacillus* spp. The comparison of the core and pan genomes towards available genomes from strains of various origins, of several isolates, which exhibit a potential for their use as starter, would be of particular interest to provide information on niche adaptation and go further on their possible tailored-application.

Declaration of Competing Interest

The authors declare no conflict of interest.

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